09/724,613

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=> file biosis medline caplus wpids uspatfull
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=> s releas? (3a) nucleic acid? (3a) surfactant
   4 FILES SEARCHED...
L1             7 RELEAS? (3A) NUCLEIC ACID? (3A) SURFACTANT

=> s l1 and protease
L2             4 L1 AND PROTEASE

=> s l2 and cationic
L3             0 L2 AND CATIONIC

=> d l2 bib abs 1-4

L2   ANSWER 1 OF 4  CAPLUS  COPYRIGHT 2002 ACS
AN   1992:3209  CAPLUS
DN   116:3209
TI   Methods of extracting nucleic acids for PCR amplification without using a
     proteolytic enzyme
IN   Cummins, Thomas J.; Ekeze, Tobias D.
PA   USA
SO   Can. Pat. Appl., 35 pp.
     CODEN: CPXXEB
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.         KIND  DATE           APPLICATION NO.   DATE
     ---------------    ----  --------       ---------------   --------
PI   CA 2025845          AA   19910419       CA 1990-2025845   19900920
     US 5231015          A    19930727       US 1989-423071    19891018
     EP 428197           A2   19910522       EP 1990-202723    19901012
     EP 428197           A3   19910529
     EP 428197           B1   19970102
         R:  AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE
     AT 147108           E    19970115       AT 1990-202723    19901012
     JP 03133379         A2   19910606       JP 1990-277920    19901018
     JP 2533969          B2   19960911
     US 5543305          A    19960806       US 1995-471806    19950606
PRAI US 1989-423071           19891018
     US 1993-10249            19930128
```

AB A rapid and highly effective method for extg. nucleic acids from cells or virions without using proteolytic enzymes consists of (1) mixing a lysing compn. comprising an org. buffer which maintains the pH at 4-10, a source of a DNA polymerase cofactor, a stabilizer, and .gtoreq.1 compatible nonionic **surfactant** which will **release nucleic acids** from cytoplasmic and nuclear membranes of cells or virions; (2) heating the resulting mixt. at or near the b.p. of water for 5-15 min; (3) recovering the nucleic acids for amplification by the polymerase chain reaction (PCR). Human immunodeficiency virus-1 (HIV-1) DNA was extd. from patient blood cells using a lysing compn. contg. Tris buffer (10 mM, pH 8.3), KCl 50, MgCl2 2.5 mM, gelatin 0.1 .mu.g/mL, Nonidet P-40 0.45, and Tween 20 0.45 wt.%. The resulting mixt. was heated at 100.degree. for 10 min and centrifuged .apprx.2 s at 14,000 rpm. Four primers were used in the amplification reaction.

L2 ANSWER 2 OF 4 USPATFULL
AN 2002:119586 USPATFULL
TI Identification of essential genes in prokaryotes
IN Haselbeck, Robert, San Diego, CA, UNITED STATES
Ohlsen, Kari L., San Diego, CA, UNITED STATES
Zyskind, Judith W., La Jolla, CA, UNITED STATES
Wall, Daniel, San Diego, CA, UNITED STATES
Trawick, John D., La Mesa, CA, UNITED STATES
Carr, Grant J., Escondido, CA, UNITED STATES
Yamamoto, Robert T., San Diego, CA, UNITED STATES
Xu, H. Howard, San Diego, CA, UNITED STATES
PI US 2002061569 A1 20020523
AI US 2001-815242 A1 20010321 (9)
PRAI US 2000-191078P 20000321 (60)
US 2000-206848P 20000523 (60)
US 2000-207727P 20000526 (60)
US 2000-242578P 20001023 (60)
US 2000-253625P 20001127 (60)
US 2000-257931P 20001222 (60)
US 2001-269308P 20010216 (60)
DT Utility
FS APPLICATION
LREP KNOBBE MARTENS OLSON & BEAR LLP, 620 NEWPORT CENTER DRIVE, SIXTEENTH FLOOR, NEWPORT BEACH, CA, 92660
CLMN Number of Claims: 44
ECL Exemplary Claim: 1
DRWN 4 Drawing Page(s)
LN.CNT 30870
AB The sequences of antisense nucleic acids which inhibit the proliferation of prokaryotes are disclosed. Cell-based assays which employ the antisense nucleic acids to identify and develop antibiotics are also disclosed. The antisense nucleic acids can also be used to identify proteins required for proliferation, express these proteins or portions thereof, obtain antibodies capable of specifically binding to the expressed proteins, and to use those expressed proteins as a screen to isolate candidate molecules for rational drug discovery programs. The nucleic acids can also be used to screen for homologous nucleic acids that are required for proliferation in cells other than Staphylococcus aureus, Salmonella typhimurium, Klebsiella pneumoniae, and Pseudomonas aeruginosa. The nucleic acids of the present invention can also be used in various assay systems to screen for proliferation required genes in other organisms.

L2 ANSWER 3 OF 4 USPATFULL
AN 96:70348 USPATFULL
TI Methods of extracting deoxyribonucleic acids without using a proteolytic enzyme
IN Cummins, Thomas J., Rochester, NY, United States

Ekeze, Tobias D., Rochester, NY, United States
PA Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY, United States (U.S. corporation)
PI US 5543305 19960806
AI US 1995-471806 19950606 (8)
RLI Continuation of Ser. No. US 1993-10249, filed on 28 Jan 1993, now abandoned which is a division of Ser. No. US 1989-423071, filed on 18 Oct 1989, now patented, Pat. No. US 5231015
DT Utility
FS Granted
EXNAM Primary Examiner: Jones, W. Gary; Assistant Examiner: Marschel, Ardin H.
CLMN Number of Claims: 15
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 887
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides a rapid and highly effective method for extracting nucleic acids from cells or virions without the use of proteolytic enzymes. Extraction is accomplished within a few minutes using a lysing composition comprising a buffer, a source of a DNA polymerase cofactor, a stabilizer and at least one nonionic **surfactant** which will **release nucleic acids** from cytoplasmic and nuclear membranes of cells or virions. The resulting mixture is heated to boiling for up to fifteen minutes, and the nucleic acids are recovered for amplification using polymerase chain reaction. No proteolytic enzyme is used in the extraction process.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 4 OF 4 USPATFULL
AN 93:61024 USPATFULL
TI Methods of extracting nucleic acids and PCR amplification without using a proteolytic enzyme
IN Cummins, Thomas J., Rochester, NY, United States
Ekeze, Tobias D., Rochester, NY, United States
PA Eastman Kodak Company, Rochester, NY, United States (U.S. corporation)
PI US 5231015 19930727
AI US 1989-423071 19891018 (7)
DT Utility
FS Granted
EXNAM Primary Examiner: Moskowitz, Margaret; Assistant Examiner: Marschel, Ardin H.
LREP Tucker, J. Lanny
CLMN Number of Claims: 15
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 811
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides a rapid and highly effective method for extracting nucleic acids from cells or virions without the use of proteolytic enzymes. Extraction is accomplished within a few minutes using a lysing composition comprising a buffer, a source of a DNA polymerase cofactor, a stabilizer and at least one nonionic **surfactant** which will **release nucleic acids** from cytoplasmic and nuclear membranes of cells or virions. The resulting mixture is heated to boiling for up to fifteen minutes, and the nucleic acids are recovered for amplification using polymerase chain reaction. No proteolytic enzyme is used in the extraction process.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=>

```
=> s l4 and protease
L5            0 L4 AND PROTEASE

=> s releas? (5a) nucleic acid? and protease?
   4 FILES SEARCHED...
L6          383 RELEAS? (5A) NUCLEIC ACID? AND PROTEASE?

=> s l6 and cationic (3a) surfactant?
L7            3 L6 AND CATIONIC (3A) SURFACTANT?

=> d l7 bib abs 1-3

L7   ANSWER 1 OF 3  USPATFULL
AN     2001:75154  USPATFULL
TI     Method for isolation DNA
IN     Gautsch, James W., Solana Beach, CA, United States
       Saghbini, Michael G., San Diego, CA, United States
       Lippman, David A., San Marcos, CA, United States
       Dana, Richard C., Escondido, CA, United States
PA     Bio101, Inc., Carlsbad, CA, United States (U.S. corporation)
PI     US 6235501          B1   20010522
AI     US 1997-937905           19970925 (8)
RLI    Continuation of Ser. No. US 1995-388504, filed on 14 Feb 1995, now
       abandoned
DT     Utility
FS     Granted
EXNAM  Primary Examiner: Prats, Francisco
LREP   Fitting, Thomas
CLMN   Number of Claims: 37
ECL    Exemplary Claim: 1
DRWN   14 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 1576
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     The invention describes a method for the isolation of components from
       samples, particularly large molecular weight DNA from biological
       samples. The method involves the application of controlled oscillatory
       mechanical energy to the sample for short periods of time of about 5 to
       60 seconds to lyse the sample and release the component(s) from the
       sample, followed by standard isolation methods. In preferred
       embodiments, the method includes the use of a spherical particle for
       applying the mechanical energy.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7   ANSWER 2 OF 3  USPATFULL
AN     2001:1610  USPATFULL
TI     Methods and devices for collecting and storing clinical samples for
       genetic analysis
IN     Harvey, Michael A., Spofford, NH, United States
       Kremer, Richard D., Keene, NH, United States
       Burghoff, Robert L., Westmoreland, NH, United States
       King, Thomas H., Brattleboro, VT, United States
PA     Schleicher & Schuell, Inc., Keene, NH, United States (U.S. corporation)
PI     US 6168922          B1   20010102
AI     US 1999-255151           19990222 (9)
RLI    Continuation of Ser. No. US 1997-835614, filed on 9 Apr 1997, now
       patented, Pat. No. US 5939259
DT     Utility
FS     Granted
EXNAM  Primary Examiner: Witz, Jean C.
LREP   Voyce, Brian D.
CLMN   Number of Claims: 34
ECL    Exemplary Claim: 1
```

DRWN 7 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 551
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to devices and methods for the collection, storage, and purification of nucleic acids, such as DNA or RNA, from fluid samples for subsequent genetic characterization, primarily by conventional amplification methods. The present invention can be used to collect, store, or purify nucleic acids either from a biological source other than untreated whole blood, the biological source having naturally occurring nucleic acid amplification inhibitors present, (including either a buccal swab, cerebrospinal fluid, feces, lymphatic fluid, a plasma sample, a saliva sample, a serum sample, urine, or a suspension of cells or viruses), or from a treated whole blood source that has naturally occurring nucleic acid amplification inhibitors present, as well as added blood stabilization components that also inhibit nucleic acid amplification. More importantly, these **nucleic acids** can be **released** after collection or storage in a manner that enables them to be amplified by conventional techniques such as polymerase chain reaction. In particular, an absorbent material that does not bind nucleic acids irreversibly is impregnated with a chaotropic salt. A biological source sample is contacted with the impregnated absorbent material. Any nucleic acids present in the biological source can be either eluted or resolubilized off the absorbent material.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 3 OF 3 USPATFULL
AN 1999:96210 USPATFULL
TI Methods and devices for collecting and storing clinical samples for genetic analysis
IN Harvey, Michael A., Spofford, NH, United States
Kremer, Richard D., Keene, NH, United States
Burghoff, Robert L., Westmoreland, NH, United States
King, Thomas H., Brattleboro, VT, United States
PA Schleicher & Schuell, Inc., Keene, NH, United States (U.S. corporation)
PI US 5939259 19990817
AI US 1997-835614 19970409 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Witz, Jean C.; Assistant Examiner: Hanley, Susan
LREP Voyce, Brian D.
CLMN Number of Claims: 12
ECL Exemplary Claim: 1
DRWN 7 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 471
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to devices and methods for the collection, storage, and purification of nucleic acids, such as DNA or RNA, from fluid samples for subsequent genetic characterization, primarily by conventional amplification methods. The present invention can be used to collect, store, or purify nucleic acids from a treated whole blood source that has naturally occurring nucleic acid amplification inhibitors present, as well as added blood stabilization components that also inhibit nucleic acid amplification. More importantly, these **nucleic acids** can be **released** after collection or storage in a manner that enables them to be amplified by conventional techniques such as polymerase chain reaction. In particular, an absorbent material that does not bind nucleic acids irreversibly is impregnated with a chaotropic salt. A biological source sample is contacted with the impregnated absorbent material. Any nucleic acids present in the biological source can be either eluted or resolubilized off the absorbent material.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l7 kwic 1-3

L7 ANSWER 1 OF 3 USPATFULL

DETD . . . the components of the liquid medium under sufficient mechanical energy of an oscillatory nature to lyse the tissue components and **release nucleic acids**.

DETD . . . involves subjecting the source material to mechanical force and energy that disrupts the cells with violent impact action with consequent **release** of the **nucleic acids**. The **released** DNA or RNA then is recovered, e.g., from a liquid phase of the starting material, such procedure being known in. . .

DETD . . . impart impact energy to the beads and these strike the source material cells repeatedly to open the cells so the **nucleic acids** can be **released**.

DETD In dealing with the quest for improving mechanical lysing of tissues for **release** of cellular components, particularly **nucleic acids**, it is seen that an apparatus that allows simultaneous separation of plural samples at very high oscillating rate while maintaining. . .

DETD . . . to a source material of up to about 450 times gravity (.times.g) or more thereby producing relatively complete lysis and **release** of **nucleic acids** in a time period that can be as low as from about 3 seconds to about 5 minutes where a. . .

DETD . . . acceleration that will produce sufficient mechanical energy in the source material that produces the cell disruption or fracture to allow **release** of **nucleic acids** from the organized structures of the cells of the tissue.

DETD . . . magnitudes as they collide with the cells of nucleic acid source material therein and produce significant cell disruption to allow **nucleic acids** to **release** from the cells.

DETD . . . through the pores during the rupturing process to the extent of the pore diameter. This embodiment facilitates separation of the **released** suspension, including **nucleic acids** from insoluble or indestructible materials in the tissue. In this embodiment, the outer container collects the material which passed out. . .

DETD . . . to mechanical energy of a particular type as specified herein so as to disrupt tissue and cell structure sufficiently to **release nucleic acids**, and particularly DNA, into the liquid phase for subsequent recovery and purification.

DETD . . . may be isolated from a disrupted mixture containing an extraction solvent that comprises a neutral buffer and a cocktail of **protease** inhibitors.

DETD . . . applied to the tissues, and provides a means for impacting, striking, breaking and/or rupturing the tissue so as to facilitate **release** of **nucleic acids** from the tissue and the DNA isolation process.

DETD . . . and also on the particular tissue being treated, with the end objective of selecting a mechanical lysing force sufficient to **release nucleic acid** without compromising the quality of the recovered product.

DETD . . . conditions are capable of generating enough mechanical energy by reciprocal motion to break the tissue structure and cell walls and **release** the **nucleic acids**.

DETD The selected detergent may be any of a variety of conventional **surfactants** including anionic, **cationic**, non-ionic and amphoteric surfactants.

DETD D. **Protease** Solution: 5 mg/ml Proteinase K, 5 mg/ml Pronase in Cell Resuspension Solution with 1% SDS.

DETD . . . SDS previously added, and the mixture is incubated at 55-65

degrees Centigrade (C) for 10 minutes. Thereafter, 35 ul of **Protease** Solution is added and thoroughly mixed, and incubated at 55.degree. C. for 10 minutes, inverting occasionally. Thereafter, 450 ul of. . .

CLM What is claimed is:

1. A method of isolating high molecular weight nucleic acid from a biological material which comprises mechanically **releasing** said high molecular weight **nucleic acid** from said material by the application of rapidly oscillating reciprocal mechanical energy to said material in the presence of a liquid medium in a closed container to produce a **released** high molecular weight **nucleic acid** solution, wherein said **released** high molecular weight **nucleic acid** has an average molecular weight greater than 10 kilobases, said liquid medium contains one or more particles and detergent in. . .

28. The method of claim 1 wherein said method further comprises the step of isolating said **nucleic acid** from said **released nucleic acid** solution.

L7 ANSWER 2 OF 3 USPATFULL

AB . . . acid amplification inhibitors present, as well as added blood stabilization components that also inhibit nucleic acid amplification. More importantly, these **nucleic acids** can be **released** after collection or storage in a manner that enables them to be amplified by conventional techniques such as polymerase chain. . .

SUMM . . . acid amplification inhibitors present, as well as added blood stabilization components that also inhibit nucleic acid amplification. More importantly, these **nucleic acids** can be **released** after collection or storage in a manner that enables them to be amplified by conventional techniques such as polymerase chain. . .

SUMM . . . fluids when one desires to analyze any nucleic acids present in the biological source. One would have to use a **protease** digestion, organic extraction, and/or an ion exchange step in order to retrieve nucleic acids.

SUMM . . . used to detect pathogens such as bacteria or viruses that can be found in the circulatory system. More importantly, these **nucleic acids** can be **released** after collection or storage in a manner that enables them to be amplified by conventional techniques such as polymerase chain reaction. The **release** of amplifiable **nucleic acids** is substantially more than in the presence of the inhibitory composition alone. In particular, an absorbent material that does not. . .

SUMM . . . damage either nucleic acid templates, (such as RNAses, DNAses), or damage PCR polymerases used in the amplification reactions, (such as **proteases**). These substances can be found in buccal swabs, cerebrospinal fluids, feces, lymphatic fluids, plasma, saliva, serum, sputum, or urine. In. . .

DETD . . . order to enhance lysis or disruption of intact cells, bacteria or viruses absorbed onto the device. For example, suitable anionic, **cationic**, or zwitterionic **surfactants**, such as Tween 20 or Triton X-100, can be impregnated into the absorbent material along with the chaotropic salt. Suitable. . .

CLM What is claimed is:

. . . comprising an absorbent material that does not bind irreversibly to nucleic acids, a chaotropic salt impregnated about the absorbent material, **nucleic acids** from the biological source **releasably** bound to the absorbent in an amplifiable form, the amplification inhibitors being bound to the adsorbent such that resolubilization does not occur by **releasing** the **nucleic acids** from the adsorbent.

. . . and the amplification inhibitors from the biological source being bound to the adsorbent such that resolubilization does not occur by **releasing** the **nucleic acids** from the adsorbent.

L7 ANSWER 3 OF 3 USPATFULL

AB . . . acid amplification inhibitors present, as well as added blood stabilization components that also inhibit nucleic acid amplification. More importantly, these **nucleic acids** can be **released** after collection or storage in a manner that enables them to be amplified by conventional techniques such as polymerase chain. . .

SUMM . . . acid amplification inhibitors present, as well as added blood stabilization components that also inhibit nucleic acid amplification. More importantly, these **nucleic acids** can be **released** after collection or storage in a manner that enables them to be amplified by conventional techniques such as polymerase chain. . .

SUMM . . . fluids when one desires to analyze any nucleic acids present in the biological source. One would have to use a **protease** digestion, organic extraction, and/or an ion exchange step in order to retrieve nucleic acids.

SUMM . . . used to detect pathogens such as bacteria or viruses that can be found in the circulatory system. More importantly, these **nucleic acids** can be **released** after collection or storage in a manner that enables them to be amplified by conventional techniques such as polymerase chain reaction. The **release** of amplifiable **nucleic acids** is substantially more than in the presence of the inhibitory composition alone. In particular, an absorbent material that does not. . .

SUMM . . . damage either nucleic acid templates, (such as RNAses, DNAses), or damage PCR polymerases used in the amplification reactions, (such as **proteases**). These substances can be found in buccal swabs, cerebrospinal fluids, feces, lymphatic fluids, plasma, saliva, serum, sputum, or urine. In. . .

DETD . . . order to enhance lysis or disruption of intact cells, bacteria or viruses absorbed onto the device. For example, suitable anionic, **cationic**, or zwitterionic **surfactants**, such as Tween 20 or Triton X-100, can be impregnated into the absorbent material along with the chaotropic salt. Suitable. . .

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=> file biosis medline caplus wpids uspatfull

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FULL ESTIMATED COST	0.21	0.21

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=> s releas? nucleic acid? and inhibitor
L1 43 RELEAS? NUCLEIC ACID? AND INHIBITOR

=> s l1 and ribonuclease
L2 4 L1 AND RIBONUCLEASE

=> d l2 bib abs 1-4

L2 ANSWER 1 OF 4 USPATFULL
AN 2000:124763 USPATFULL
TI Pressure-enhanced extraction and purification
IN Laugharn, Jr., James A., Winchester, MA, United States
Hess, Robert A., Cambridge, MA, United States
Tao, Feng, Boston, MA, United States
PA BBI BioSeq, Inc., Woburn, MA, United States (U.S. corporation)
PI US 6120985 20000919
AI US 1998-83651 19980522 (9)
RLI Continuation-in-part of Ser. No. US 1998-16062, filed on 30 Jan 1998 which is a continuation-in-part of Ser. No. US 1997-962280, filed on 31 Oct 1997
DT Utility
FS Granted
EXNAM Primary Examiner: Wilson, James O.
LREP Fish & Richardson P.C.
CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN 17 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 2180
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods for cell lysis and purification of biological materials, involving subjecting a sample maintained at a subzero temperature to high pressure, are disclosed. Apparatus for practicing the methods are also disclosed. The cell or cells that are lysed may be in suspension or part of a tissue. They are lysed by a method that includes: (i) providing a frozen cell or cells under atmospheric pressure; (ii) while maintaining the cell or cells at a subzero temperature, exposing the cell or cells to an elevated pressure in a pressure chamber, the

elevated pressure being sufficient to thaw the frozen cell or cells at the subzero temperature; (iii) depressurizing the pressure chamber to freeze the cell or cells at the subzero temperature; and (iv) repeating the exposing and depressurizing steps until the cell or cells are lysed. This method can lyse a cell or cells with or without cell walls; such cells include, but are not limited to, bacteria, viruses, fungal cells (e.g, yeast cells), plant cells (e.g, corn leaf tissue), animal cells, insect cells, and protozoan cells.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 2 OF 4 USPATFULL
AN 2000:57541 USPATFULL
TI Reagent and method for isolation and detection of selected nucleic acid sequences
IN Summerton, James E., Corvallis, OR, United States
Weller, Dwight D., Corvallis, OR, United States
Wages, Jr., John M., Pacifica, CA, United States
PA AVI BioPharma, Inc., Corvallis, OR, United States (U.S. corporation)
PI US 6060246 20000509
AI US 1997-969813 19971113 (8)
PRAI US 1996-30963P 19961115 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Jones, W. Gary; Assistant Examiner: Whisenant, Ethan
LREP LeeAnn Gorthey Dehlinger & Associates
CLMN Number of Claims: 18
ECL Exemplary Claim: 1
DRWN 9 Drawing Figure(s); 9 Drawing Page(s)
LN.CNT 1477
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to compositions and methods for rapidly detecting or isolating a target nucleic acid sequence in a polynucleotide-containing sample. The sample is exposed to a rapid pairing reagent, which contains a rapid capture component, effective to rapidly and non-selectively bind polynucleotides, and a target specific probe, effective to selectively bind the target nucleic acid sequence. Selectively disrupting the binding between the capture component and polynucleotides leaves only target sequence bound to the rapid pairing reagent.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 3 OF 4 USPATFULL
AN 95:18342 USPATFULL
TI Non toxic compositions and methods useful for the extraction of nucleic acids
IN Ness, Jeffrey V., Bothell, WA, United States
Cimler, B. Melina, Portland, OR, United States
Meyer, Jr., Rich B., Woodinville, WA, United States
Vermeulen, Nicolaas M. J., Woodinville, WA, United States
PA MicroProbe Corporation, Bothell, WA, United States (U.S. corporation)
PI US 5393672 19950228
AI US 1993-156519 19931123 (8)
RLI Continuation of Ser. No. US 1992-900379, filed on 17 Jun 1992, now abandoned which is a continuation of Ser. No. US 1991-649389, filed on 1 Feb 1991, now patented, Pat. No. US 5130423 which is a continuation-in-part of Ser. No. US 1990-552745, filed on 13 Jul 1990, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Geist, Gary
LREP Townsend and Townsend Khourie and Crew
CLMN Number of Claims: 25

ECL Exemplary Claim: 1
DRWN 2 Drawing Figure(s); 1 Drawing Page(s)
LN.CNT 930
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to safe and effective methods for the extraction of nucleic acids. In particular, methods are described for isolating nucleic acid from a sample containing a biological mixture of nucleic acids and other biological compounds wherein the sample is combined with an extraction solution containing at least one organic compound such as benzyl alcohol or a benzyl alcohol derivative to form an aqueous and non-aqueous phase. The nucleic acid is isolated from the aqueous phase. Preferably, the resulting combined solution also contains bentonite, as defined below. Typically, the sample will first be combined with a lysing agent before extraction. The lysing agents preferred are chaotropic salts such as guanidinium hydrochloride and guanidinium isothiocyanate.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 4 OF 4 USPATFULL
AN 92:57788 USPATFULL
TI Non-corrosive compositions and methods useful for the extraction of nucleic acids
IN Van Ness, Jeffrey, Bothell, WA, United States
Cimler, B. Melina, Portland, OR, United States
Meyer, Jr., Rich B., Woodinville, WA, United States
Vermeulen, Nicolaas M. J., Woodinville, WA, United States
PA MicroProbe Corporation, Bothell, WA, United States (U.S. corporation)
PI US 5130423 19920714
AI US 1991-649389 19910201 (7)
RLI Continuation-in-part of Ser. No. US 1990-552745, filed on 13 Jul 1990, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Wax, Robert A.; Assistant Examiner: Bugaisky, G.
LREP Leith, Debra
CLMN Number of Claims: 13
ECL Exemplary Claim: 1
DRWN 2 Drawing Figure(s); 1 Drawing Page(s)
LN.CNT 818
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to safe and effective methods for the extraction of nucleic acids. In particular, methods are described for isolating nucleic acid from a sample containing a biological mixture of nucleic acids and other biological compounds wherein the sample is combined with an extraction solution containing at least one organic compound such as benzyl alcohol or a benzyl alcohol derivative to form an aqueous and non-aqueous phase. The nucleic acid is isolated from the aqueous phase. Preferably, the resulting combined solution also contains bentonite, as defined below. Typically, the sample will first be combined with a lysing agent before extraction. The lysing agents preferred are chaotropic salts such as guanidinium hydrochloride and guanidinium isothiocyanate.

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L2 ANSWER 1 OF 4 USPATFULL
SUMM . . . only solubilizes the plasma membrane (e.g., hypotonic nonidet P-40 lysis buffer). The latter reagents also require addition of a nuclease **inhibitor**. Organic solvent extraction or silica membrane absorption methods are then be used to extract the RNA from the

cell lysate.. . .

DETD **Ribonuclease** A is subject to cold-denaturation at elevated pressures. Addition of a reducing agent can subsequently facilitate irreversible denaturation by reducing. . .

DETD After treatment, each sample was centrifuged at 10,000 rpm for 10 minutes to separate **released nucleic acids** from cell debris and intact cells. Both the supernatant and the pellet were treated with proteinase K and analyzed by. . .

L2 ANSWER 2 OF 4 USPATFULL

DETD . . . polyamine may be used as a reporter group, as described below. In this case, the weakly basic amine component should **release nucleic acids** at a pH where strongly-basic amines still effectively bind nucleic acids, typically about pH 9 to 10.

DETD Generally, before utilizing the method of the invention, a specimen must be treated in order to **release nucleic acids** from biological structures. This can be accomplished by a variety of methods known in the art. Preferably, the cellular, subcellular,. . .

DETD . . . mM MnCl.sub.2, 150 mM NaCl, 0.5 mM Atp, 50 .mu.g/ml acetylated bovine serum albumin, 2 mM dithiothreitol, 60 Units recombinant **ribonuclease inhibitor** (rRNasin, Promega Biotech, Madison, Wis.), approximately 37.5 .mu.g of radiolabelled RNA transcript (prepared as above), and 30 Units poly A. . .

L2 ANSWER 3 OF 4 USPATFULL

SUMM . . . as phenol or phenol/chloroform. Chirgwin et al., Biochemistry 18:5294-5299 (1979) described the isolation of intact RNA from tissues enriched in **ribonuclease** by homogenization in guanidinium thiocyanate and 2-mercaptoethanol, followed by ethanol precipitation or by sedimentation through cesium chloride.

SUMM Further, the use of chaotropic agents such as guanidinium thiocyanate (GnSCN) are widely used to lyse and **release nucleic acid** from cells into solution, largely due to the fact that chaotropic salts inhibit nucleases and proteases. However, it has proved. . .

SUMM . . . the starting material is complex, such as feces or blood. In 1959, Brownhill et al. reported that bentonite was an **inhibitor** of nucleases (Brownhill et al., Biochem. J. 73:434 (1959)). Fraenkel-Conrat et al. later developed a procedure for the use of bentonite to inhibit **ribonuclease** in a procedure to purify tobacco mosaic virus (Fraenkel-Conrat et al., Virology 14:54-58 (1961)). Subsequent researchers reported the use of bentonite in combination with phenol and chloroform in the reduction of **ribonuclease** activity during the isolation of RNA (Jacoli et al., Can. J. Biochem. 51:1558-1565 (1973); Griffin et al., Anal. Biochem. 87:506-520. . .

DETD Also preferably resident in the extraction solution is a nuclease **inhibitor**, preferably an organoclay or the like, and more preferably bentonite, Macaloid.RTM., Bentone.RTM. (a bentonite or hectorite organoclay platelet having a. . . for extended periods of time, as compared to bentonite and Macaloid.RTM.. It may be necessary to first purify the nuclease **inhibitor**, for instance bentonite or Macaloid.RTM., as described in Sambrook et al., Molecular Cloning--A Laboratory Manual, Cold Spring Harbor Laboratory, Cold. . . small uniform particles be used. Such nuclease inhibitors are particularly desirable when extraction of RNA is desired. In samples where **ribonuclease** does not substantially hinder extraction, or if DNA only is being extracted, nucleic acid may be extracted without the use. . .

DETD . . . No. 0 127 327, which is incorporated by reference herein. The chaotropic agent is present at a concentration sufficient to **release nucleic acid** from target cells and to protect the **released nucleic acid** from nucleases. Typically, the chaotrope is present at a concentration from about 1 M to about 5 M, and more. . .

CLM What is claimed is:
1. An extraction composition for the isolation of **released nucleic acids** comprising: at least one organic compound which is a liquid at room temperature and which is selected from the group. . .
13. An extraction composition for the isolation of **released nucleic acids** comprising: a clay or a silicate or an admixture of said clay and said silicate; and at least one organic. . .

L2 ANSWER 4 OF 4 USPATFULL

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